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CHEMOANALYTICAL INVESTIGATION BY FATTY ACID PAPER CHROMATOGRAPHY IN SOME TISSUES

by

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I. INTRODUCTION

Since the study of HIRDITCH in 1947, it is generally recognized that the dépot fat of each species has a specific constitution. This fact of dépot fat is not able to be explained completely by reason of habit by which each species is fond of to eat constant food in constitution. But it is considered that each species synthesizes a specific fat in its body, as it has been believed that each species manufactures a specific protein from the contant food. However it has also been known previously that the specific character of dépot fat in each species is not always constant.

For example, if a hungry dog is given a diet, which contains the subcutaneous fat of a sheep, his dépot fat become to show a higher melting point than that of normal dogs. And since LONGENECKER (1939) followed up the change of iodine value in dépot fat by giving corn oil to fasting rats, there were many literatures concerning the change of dépot fat constitution by giving different kinds of food to various animals. In short, it does not seem so difficult to change the normal constitution of the dépot fat of an animal by giving fat more than certain extent, which is not usually given to the animal.

According to the recent experimental results by SCHOENHEIMER and RITTENBERG using deuterium and radioisotope, it is believed that dépot fat is not stored, in stationary state, as it had been formerly considered, but it is changing constantly and dynamically. And so half of the dépot fat of an animal seems to be renewed in 6 or 7 days.

Adipose tissue has been shown to contain the desaturating enzyme of fatty acids by SHAPIRO (1943), QUAGLIARIELLO (1947) and many other investigaters. Already it has been shown that compound chemical changes, such as lengthening and shortening of the carbon chain or desaturating in the part of $C^{8:9}$, are performed in adipose tissue according to SCHOENHEIMER's experiments using various kinds of fatty acids containing deuterium.

On the other hand, it is almost clear that liver fat has many close relationships to dépot fat considering the role which the liver plays in fat metabolism.

Therefore, when the dynamics of dépot fat are discussed, liver fat should be analyzed at the same time. The analysis of fatty acid constitution in fatty liver especially seems to suggest many interesting problems.

First of all, when dépot fat is treated, variations from different parts of the

whole body must be shown, and at the same time it is necessary to differentiate from common white adipose tissue, the special adipose tissue which formerly was called "hibernating gland" or brown fat, and to study the differences between them. From this point of view, the following experiments were performed.

It is considered logical to apply the method of NODA and HIRAYAMA's fatty acid paper chromatography as the method of an analyzing fat constitution because of the exactness and convenience of this method.

II. EXPERIMENTAL MATERIALS AND ANIMALS

A Experimental Materials

1 Oils

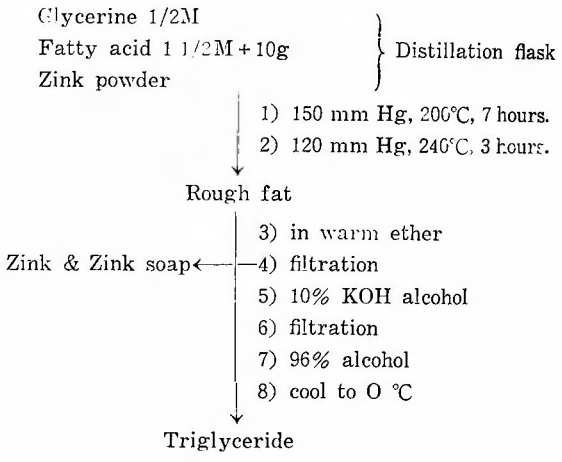
a) Sesame oil: Sesame oil of J. P. Specification (supplied by DAINIPPON Pharmaceutical Co. Ltd.).

b) Cod liver oil: refined cod liver oil.

c) Butter: Fresh butter made in Hokkaido, Japan.

d) Simple glyceride of lower fatty acids: Synthesized from pure refined glycerine, free caproic acid, caprylic acid and capric acid using zinc powder as the catalyser. The synthesizing process is shown in Table 1.

Table. 1 Synthesizing process of triglyceride



2 Other materials

a) CCl_4 solution for injection: Prepared for injection as a 5% olive oil suspension, filling aseptically up to 1 cc in each ampulle.

b) Ethionine solution for injection: Pure crystal of DL-ethionine, supplied from TAKEDA Pharmaceutical Co. Ltd., dissolved in water, and neutralized by sodium carbonate and filled aseptically up to 1 cc in each ampulle as a 2.5% DL-ethionine water solution.

c) Kerosene: Petroleum hydrocarbon was distilled on the oil bath, and distillate of 170°C~185°C in it was collected. Contaminations were removed by washing with concentrated sulfuric acid several times, then it was neutralized with sodium carbonate and the water was removed.

d) Filter paper for chromatography: "Toyo" filterpaper No. 2 was used.

e) Moving solvent of chromatography (M. A. P.).

As the moving solvent, a mixture of methanol, glacial acetic acid and kerosene (10 : 2 : 1.2 in volume) was used.

B Experimental Animals

(1) Considering the nature of experiment, healthy male rats, around 120 g

weight, were chosen as a representative omnivorous animal. (2) In the experiment of fatty liver by ethionine injection, female rats were used, because it is known that male rats do not get fatty livers from ethionine injection. (3) One group consisted of 5 rats, as a rule, in order to get a sufficient amount of fat for analysis and in order to average individual differences. (4) In the experiments of fatty liver following total pancreatectomy and analysis of adrenal gland, healthy adult dogs, weighing about 10 kg were used.

III. EXPERIMENTAL METHOD

A) Care of the Animals

1. The diet was determined from Table 2 and Table 3, consideration being given to equal calories for each group and as much fat as possible, but not so much as to lessen the appetite or cause diarrhoe.

2. The diets were labeled with low fat diet and fat diet. Fat diets were classified, according to the kind of oil contained in each diet; sesame oil diet, cod liver oil diet, butter diet and lower fatty acids diet.

Table. 2 Composition of low fat diet

	g
Flour	5.0
Maize powder	5.0
Casein	2.0
Yeast	0.5
Mc collum salt	0.2
Vitamins	0.2
cal/day	about 44 cal.
fat content	less than 3%

Table. 3 Composition of fat diet

	g
Flour	2.0
Maize powder	2.0
Casein	2.0
Yeast	0.5
Mc collum salt	0.2
Vitamins	0.2
Oil	2.5
cal/day	about 45 cal.
fat content	about 28%

3. All oils were kept as pure and fresh as possible, and mixed into the foods without cooking.

4. Animals were kept for one month under controlled conditions, with special attention being given to ventilation, temperature and cleanliness.

B) Coloring Experiment of Dépot Fat Following Per Oral Administration of Sudan III

According to NISHIMURA'S report (1956), when the rats were given sesame oil saturated with Sudan III, their white fat become pink but the brown fat was not colored at all, so it was easy to distinguish both adipose tissues.

The experiment was performed repeatedly, and the results are shown in Table 4.

The administered Sudan III was obviously absorbed not only by white but also by brown fat, but when only a little Sudan III was given it seemed to be absorbed by white fat, because the pink color is more conspicuous in white than in brown fat. When large amounts of Sudan III were given for long time, the brown fat

Table. 4 Coloring experiment of dépot fat by per oral administration of Sudan III

	after 5 days		after 15 days	
	white fat	brown fat	white fat	brown fat
A group	pink	unchanged	red	red
B group	pink	unchanged	red	red
C group	unchanged	unchanged	unchanged	unchanged

A group : rat group fed on the diet containing sesame oil saturated by Sudan III
B group : rat group fed on the diet containing alcohol saturated by Sudan III
C group : rat group fed on the diet mixed with non dissolved Sudan III

naturally became red too.

In other words, when a small amount of Sudan III was given, even if it was absorbed by brown fat, it was not clear because coloration was hindered by the orginal brown color of the fat.

And it was found that Sudan III is absorbed and deposited well in adipose tissue when it is dissolved not only in oil but also in alcohol.

Because of these facts, it is advisable to mix Sudan III into the diet a few days before killing the animals, in order to distinguish both adipose tissues.

C) Accumulation of Materials

1. Animals were sacrificed by bleeding under slight ether anaesthesia after fasting for 12 hours. Each organ was immediately taken out and divided according to Table 5, then weighed in the balance and put in BLOOR's solution.

Table. 5 Classification by divisions of materials

- white fat
  - subuctaneous fat
    - neck
    - breast (axillar, scapular, back)
    - loins (abdomen, thigh, periproctal)
  - abdominal fat
    - large omentum
    - mesenterium
    - retroperitoneal (around the kidney and genital organs)
- brown fat (axillar, scapular, paraaortic, around the kidney)
- liver

2. White fat and brown fat were easily distinguished by applying Sudan III, but if brown fat was gathered forcedly from poorly located area it seemed to be apt to error by mixing with other tissues. So brown fat was collected only from richly located area. Of course it was impossible to divide it strictly according to location, so naturally some errors could be found. But it is believed that analysis of many cases may give valuable findings, so dépot fat was divided according to Table 5.

D) Extraction and Separation of Fatty Acid

Each organ was completely ground in a basin with sea sand and was heated for 60 minutes at about 60 °C in BLOOR's solution and then in petroleum ether. The filtrate was concentrated by boiling under negative pressure, and saponification was

carried on for 3 hours by adding 1-N KOH alcohol.

After removal of the non-saponifiable matter by washing in ethyl ether and petroleum ether several times, it was made acidic with 3-N. HCl, was accumulated in ether layer, and was washed with water and evaporated ether.

The results were pure fatty acids.

E) Determination of Neutralization Value

It was determined by adding drops of 1/20-N KOH alcohol, using phenolphthalain solution as an indicator.

F) Determination of Iodine Value

It was determined by adding drops of 1/20-N. Sodium thiosulfate (WIIS's method).

G) Paper Chromatography

Paper chromatography was done by using INOUE, NODA and HIRAYAMA's method. Development was made on "TOYO" filter paper No. 2, as the parabromophenacylester 2-4-dinitrophenylhydrazone of fatty acid, with M. A. P. being used as moving solvent and kerosene being used as stationary solvent.

A starting line was penciled in 5～6 cm above the bottom of the paper, and the samples were spotted on the lines of the paper, and were uniformly sprayed with stationary solvent. The paper was rolled, and placed in the glass cylinder, which had been previously filled with the vapour of moving solvent. Then the bottom of the paper was bathed in moving solvent and the starting line was 3 cm above the surface of the solvent.

The chromatogram was developed by ascending technique at 30°C for 5～6 hours.

For the convenience of spot identification, a standard sample was developed on the same paper in parallel. The standard sample is the mixture of the derivatives of all the even numbered saturated fatty acids from C_4 to C_{20}.

Furthermore, as an identification of unsaturated acids, it was colored dark violet by spraying 0.1% diphenylcarbazone solution on the paper.

The spot identification is as follows. The number represents the carbon atoms.

4 :	butyric acid,	6 :	caproic acid,
8 :	caprylic acid,	10 :	capric acid,
12 :	lauric acid,	14 :	myristic acid,
16 :	palmitic acid,	18 :	stearic acid,
20 :	arachidic acid,	OL :	oleic acid,
LE :	linoleic acid,	LN :	linolenic acid,
DO :	docosenoic acid,	EI :	eicosenoic acid,
HX :	hexadecenoic acid,	HU :	highly unsaturated acids,
S :	standard sample.		

H) Fatty Liver Following Total Pancreatectomy

Total pancreatectomy was performed on a healthy adult male dog weighing 11.5 kg, and the dog was maintained for 36 days with insulin injections of 2 units per kg of body weight every day. Later on the dosage of insulin was reduced to

1 unit per kg of body weight every day, and the dog was sacrificed 48 days later and his liver was immediately taken out.

By reducing the insulin dosage a remarkable increase in blood sugar and intense glucosuria were found in the animal. The liver was yellowish, and even macroscopically fat accumulation was evident, a typical fatty liver was shown microscopically.

I) Acute Hepatic Injury Caused by CCl_4

A 50% CCl_4 suspension was injected intramuscularly at the rate of 0.8 cc per 100 g body weight. The animals were sacrificed by bleeding 24 hours after injection, and the liver was taken out at once. At this time also fat accumulation was seen, and the picture of typical fatty liver was shown microscopically.

J) Acute Hepatic Injury Caused by Ethionine

A 2.5% DL-ethionine solution was injected into the abdominal cavity of the animals at the rate of 75 mg per 100 g body weight.

The necessary doses of ethionine were divided into 3 parts, which were injected at two and a half hour intervals.

The animals were sacrificed by bleeding 48 hours after the initial injection and the liver was taken out. The liver also differed macroscopically from a normal one, and fat infiltration was found microscopically.

IV. RESULTS

A) Analysis of Each Oil Used in the Experiments

The neutralization value and iodine value of the various oils used in the experiments are shown on Table 6.

Table 6. Analysis of various oils using in the experiments

	neutralization value	iodine value
sesame oil	189.0	117.5
cod liver oil	185.6	154.5
butter	231.9	35.8
simple glyceride mixture of lower fatty acids	322.0	0

The high neutralization value of butter suggests the existence of many lower fatty acids in the compositions of butter. Also the high iodine value of cod liver oil shows that it contains considerable amounts of unsaturated fatty acids.

The neutralization value of a simple glyceride mixture of lower fatty acids is naturally high, and is evident that it contains no unsaturated elements which means that iodine value is zero.

The chromatographic analysis of these oils is seen in Fig. 1 and Fig. 2. Sesame oil contains common higher saturated fatty acids, such as myristic, palmitic, stearic and oleic acid, and furthermore contains large amounts of essential fatty acids (such as linoleic acid and linolenic acid), which are recently proving to be important for body nutrition.

On the other hand, cod liver oil, adding to these components, clearly contains large amounts of highly unsaturated acids (these having more than 4 double bonds), docosenoic acid and eicosenoic acid.

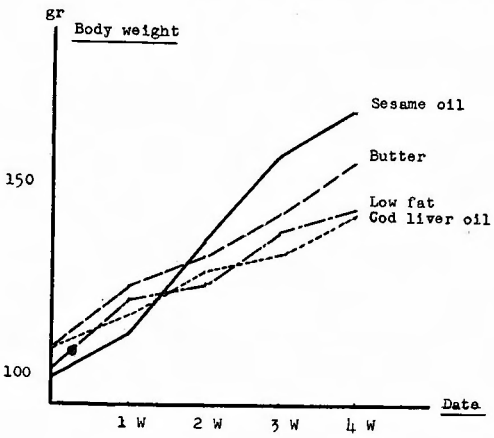
The characteristic components of butter are the lower fatty acids, such as

butyric, caproic, caprylic and capric acid. Therefore, the facts, which are presumed by neutralization value and iodine value, were clearly proven by chromatographic analysis.

B) Comparison of Body Weight Increased by Various Diet Groups

The increasing curve of average body weight from various diet groups is shown in Fig. 3.

Fig. 3 Curve of body weight in various diet group



It is difficult to draw a definite conclusion from the curve of increased body weight because it is compoundly related to the appetite of animals which may be affected by the odor of the oils contained in the food and other conditions, related to the care of the animal. But it is clear that increased body weight of sesame oil diet group is excellent and this fact agrees with our colleague's results.

An increase in body weight of low fat diet group is not inferior to other fat diet groups after keeping them 1 month.

C) Findings about the Location of Various Adipose Tissues and Their Fatty Acid Content

In Table 7, the average weights of various adipose tissues are presented in percentage to these total body weight.

Table. 7 The rate of various adipose tissues and liver to total body weight (%)

	white fat						brown fat	liver
	subcutaneous fat			abdominal fat				
	neck	breast	loins	large omentum	mesenterium	retroperitoneal		
low fat diet	0.68	1.17	2.89	0.56	1.15	2.68	0.64	4.04
sesame oil diet	0.74	1.52	3.51	0.57	1.50	4.28	0.82	4.91
cod liver oil diet	0.39	1.15	2.96	0.46	1.20	3.54	0.66	4.40
butter diet	0.47	1.44	3.22	0.45	1.17	3.45	0.76	4.27

In comparison with other groups, the highest development of dépot fat was found in the sesame oil diet group. And so it is presumed that an excellent

increase in body weight in the sesame oil diet group depends chiefly on an increase of dépot fat.

The best development of dépot fat was seen at the loin and in the retroperitoneal area of animals in all groups.

It is interesting that the total weight of brown fat shows a fairly constant rate in comparison to body weight. On the contrary, the location of white fat shows considerable individual difference.

According to these facts, evidence seems to be given to the opinion that brown fat is not simple dépot fat but a special organ having a definite function. Throughout all groups, the weight ratio of white fat to brown fat is 93 : 7.

The content of neutral fat in various adipose tissues and the liver is shown on Table 8. Neutral fat is the richest in abdominal fat and the poorest in brown fat. Brown fat is richer in many components except neutral fat than white fat, which agrees with FAWCETT's opinion that "white fat is richer only in neutral fat".

D) Analysis of Dépot Fat and Liver Fat of Low-Fat Diet Group

Analysis was done on various adipose tissues and the livers of animals, after feeding them a low-fat diet for 1 month.

A comparison of neutralization value and iodine value is shown in Table. 9. In this Table it is characteristic that no remarkable differences are found between the

Table. 8 Neural fat content in various adipose tissues in low fat diet group

	%
subcutaneous fat	45.35
abdominal fat	55.43
brown fat	24.78
liver	2.76

Table. 9 Analysis of various adipose tissues and liver in low fat diet group

	neutralization value	iodine value
subcutaneous fat	196.2	71.9
abdominal fat	198.9	68.2
brown fat	197.5	67.8
liver	165.3	148.5

kinds of adipose tissue. Liver fat shows a considerable low neutralization value and a very high iodine value. In this instance, chromatographic analysis (Fig. 4) showed that dépot fat contains common higher fatty acids such as lauric, myristic, palmitic, stearic, oleic and hexadecenoic acid and essential fatty acids. No particular differences were found between the kinds of adipose tissue.

On the contrary, in liver fat, myristic, oleic and hexadecenoic acid appear less often than in dépot fat, and lauric acid was scarcely found, but there was a considerable amount of highly unsaturated fatty acid, which was not found in dépot fat at all. These facts have been distinctly indicated by the neutralization values and iodine values.

In a word, considerable difference between dépot fat and liver fat is found, but differences cannot be found between various locations and kinds of adipose tissue by means of these analysis.

And another interesting fact is that it seems to be synthesized chiefly from carbohydrate in the daily food.

E) Analysis of Dépot Fat and Liver Fat of Various Fat Diet Groups

The same analysis were performed on the animals of various fat diet groups. A change of neutralization value is shown in Table 10 and Fig. 5. Also a change of iodine value is shown in Table 11 and Fig. 6.

Table. 10 Change of neutralization value in various diet group

	white fat		brown fat	liver
	subcut. fat	abdom. fat		
low fat diet	196.2	198.9	197.5	165.3
sesame oil diet	193.7	194.6	195.3	173.4
cod liver oil diet	190.5	192.5	191.7	170.1
butter diet	205.5	207.2	203.3	173.8

Table. 11 Change of iodine value in various diet group

	white fat		brown fat	liver
	subcut. fat	abdom. fat		
low fat diet	71.9	68.2	67.8	148.5
sesame oil diet	105.2	107.1	103.5	151.2
cod liver oil diet	89.0	90.0	87.6	158.9
butter diet	63.8	64.4	61.7	146.6

Fig. 5

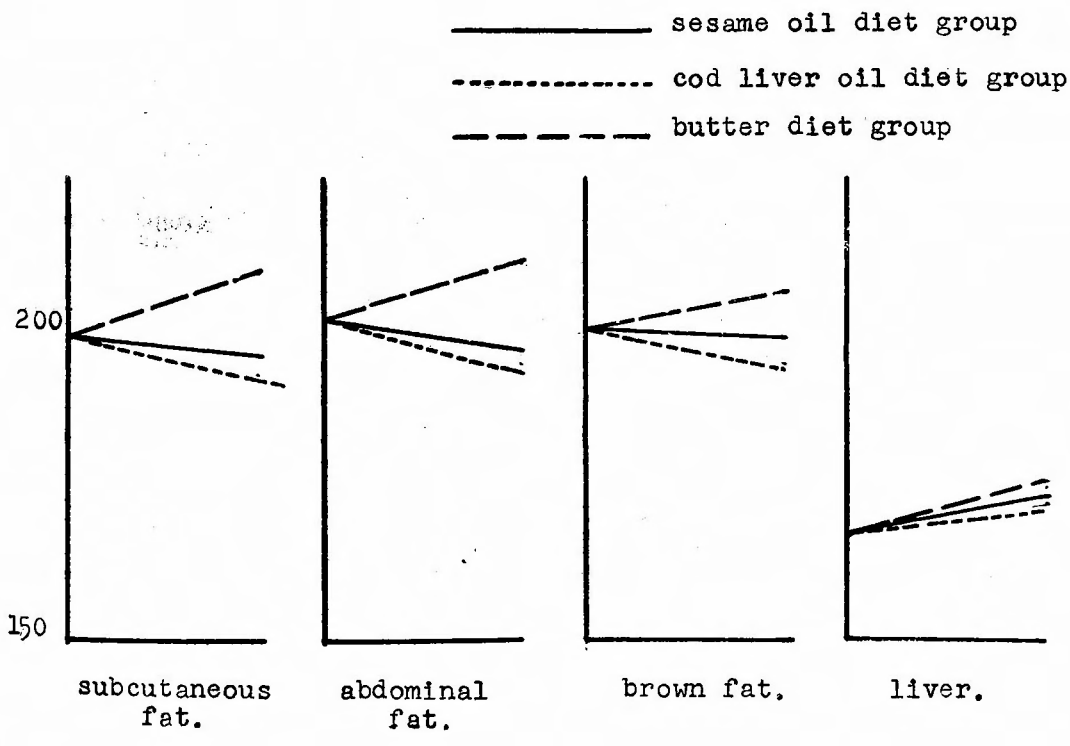
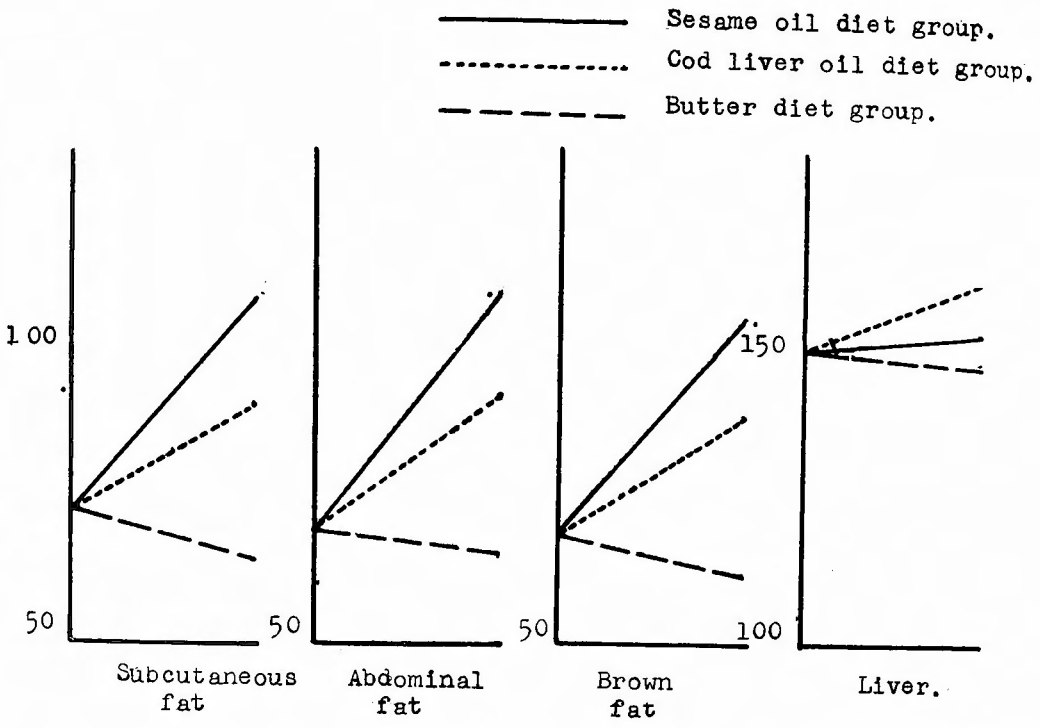


Fig. 6



A comparison of the neutralization values in dépot fat shows that the butter diet group had the highest value. The sesame oil diet group and cod liver oil diet group followed in order, but almost no changes were found in liver fat.

Nevertheless, an increase of the neutralization value of dépot fat in butter diet seems to be comparatively little, though the food contains much butter, the neutralization value of which is more than 230.

As to change of iodine value in dépot fat, the sesame oil diet group showed the greatest increase, the cod liver oil diet group and butter diet group followed it.

It seems illogical that the dépot fat of the cod liver oil diet group is found to have a lower iodine value than that of the sesame oil diet group, considering the fact that cod liver oil itself has a higher iodine value than sesame oil.

And as to the liver fat, iodine value of each group was in the order of the iodine value of the oil contained in the diet. And throughout the all groups, it should not be missed that the iodine values of brown fat have a tendency to show a lower value than that of white fat.

In order to explain these facts logically, the following chromatogram, which analyzed all the adipose tissues and the liver fat of various fat diet groups, is necessary.

Fig. 7 is the chromatogram of the dépot fat and liver fat of the sesame oil diet group, in which in comparison with low fat diet group no changes of fatty acid composition were found except an increase of linoleic acid and linolenic acid.

The remarkable increase of iodine value, in the dépot fat of the sesame oil diet

group, may be due to the smooth transportation and deposition of linoleic acid and linolenic acid which are the chief components of sesame oil.

From the view-point of resemblance of composition between sesame oil and dépot fat, it is easily presumed that absorption, transportation and deposition of these components are smoothly performed. Chromatographic analysis in the cod liver oil diet group is shown in Fig. 8, in which we can find clearly that the increase of a highly unsaturated acid, docosenoic acid and eicosenoic acid were found in the liver fat, but these acids showed only a faint shadow in the chromatogram of dépot fat. For the following reason the iodine value of the cod liver oil diet group was not so high in dépot fat but higher than the sesame oil diet group in liver fat. The important components of cod liver oil such as highly unsaturated acid, docosenoic acid and eicosenoic acid were deposited in the liver accumulated, and therefore scarcely transported to dépot fat.

The chromatographic analysis of the butter diet group is shown in Fig. 9. In this chromatogram, a proportional increase of myristic acid and lauric acid in dépot fat is comparable to an increase of the neutralization value of the group, but any trace of the deposition of lower fatty acids (less than C_{10}), which are characteristic components of butter, was not found in dépot fat. Furthermore in liver fat, not even an increase of myristic acid and lauric acid were found.

In other words, it is considered that these lower fatty acids could not stay in a living body for a long time by treating and burning immediately after absorption.

But on the other hand, according to other research on the fatty acid composition of butter, it contains only 1～2% of these lower fatty acids. So it seems to be too early to draw conclusions about the movements of the lower fatty acids by experiments using a butter diet. The following experiment was done.

F) Analysis of Dépot Fat and Liver Fat of the Lower Fatty Acids Diet Group

In order to do research on the movements of lower fatty acids in a living body, it is necessary to give continuously a large amount of these acids. But natural fats are not suitable for this experiment, so one must be synthesized artificially.

Free fatty acids can be received in many places but they are too stimulous to give animals because of their high acidity. Then non-stimulative triglyceride was synthesized by the method shown on Table 1, and this simple glyceride mixture of equal dosages of tricaproin, tricaprylin, and tricaprin was added to the animal's food for 15 days. The same analysis was done for dépot fat and liver fat. The alternation of neutralization value is shwn on Table 12 and Fig. 10. In this case, no distinct changes were seen in liver fat, but on the contrary in the dépot fat an increase of neutralization value and a decrease of iodine value were found daily. In the chromatogram (Fig. 12), we could recongnize an appearance of the lower fatty acids in dépot fat even after 5 days feeding, and distinct deposition of these acids was seen after 15 days feeding.

In other words, it was clarified that if these lower fatty acids are given sufficiently and continually, they are also transported into dépot fat and deposited there, and they are not found in liver fat even 12 hours after feeding because of rapid

oxidation.

G) Analysis of Liver Fat in Cases of Acute Hepatic Injury

Through previous experiments, some findings on the effects of exogenous fat on dépot fat and liver fat of animals were obtained. But another experiment seemed

Fig. 10

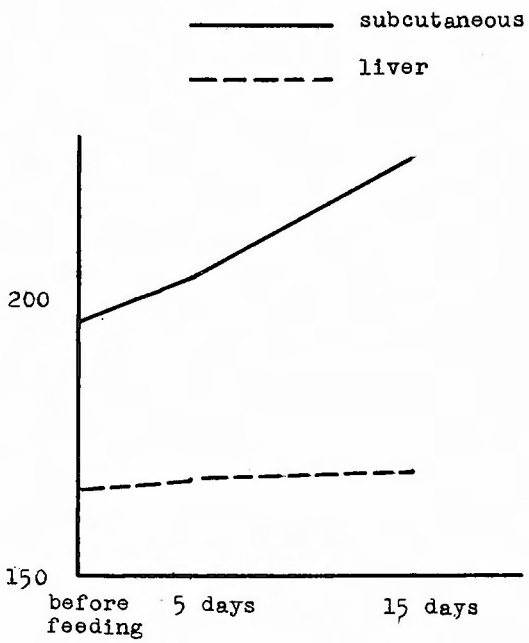


Fig. 11

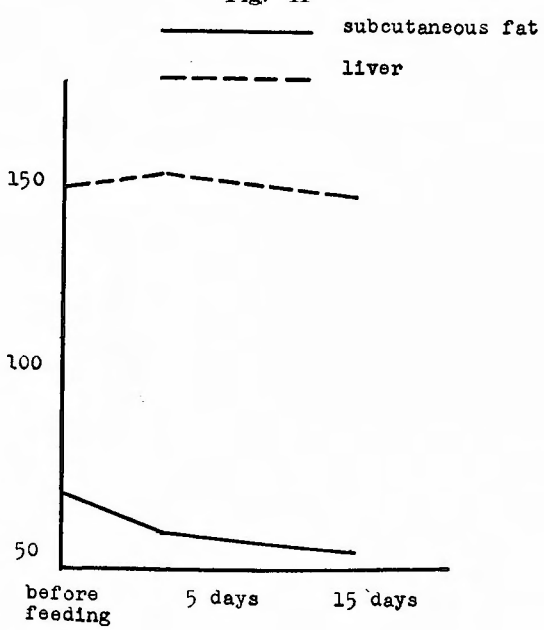


Table. 12 Change of neutralization value in lower fatty acids diet group

	before	after 5 days	after 15 days
subcutaneous fat	196.2	204.6	226.0
liver	165.3	167.7	168.3

Table. 13 Change of iodine value in lower fatty acids diet group

	before	after 5 days	after 15 days
subcutaneous fat	71.9	60.6	56.5
liver	148.5	150.2	146.4

to be necessary to understand the mechanism of dépot fat mobilization.

Previously a close relationship between dépot fat and liver fat was mentioned. It is presumed that the mechanism of fat mobilization may be clarified to certain extent by means of the analysis of liver fat in various cases of hepatic injury or in the case of a fatty liver, so the following experiments were performed.

1) The Analysis of a Fatty Liver Following Total Pancreatectomy

As above mentioned, the dog was maintained with insulin injection after total pancreatectomy and suffered from diabetes by reducing the insulin dosis, and sacrificed 48 days after operation.

The analysis of the liver fat is presented in Table 14 and Fig. 16. The fatty liver, as it has been observed macroscopically and microscopically, contained 10 times the amount of fat, in comparsion with a normal liver. And increase of neutralization value and decrease of iodine value were characteristic findings.

In comparison with a normal liver by means of chromatography (Fig. 16), a fatty liver showed a distinct increase of oleic acid and hexadecenoic acid, a considerable increase of common saturated higher acids, an appearance of lauric acid and also a clear decrease of highly unsaturated fatty acids. Referring to the chromatogram of the dépot fat of a normal dog (Fig. 14), the fatty acid composition of a fatty liver clearly resembles that of dépot fat.

Table. 14 Analysis of fatty liver following total pancreatectomy (dog)

	fat content %	neutralization value	iodine value
normal liver	0.8 ~ 2.1%	160 ~ 176	124 ~ 151
fatty liver	24.6%	187	95.5

Table. 15 Analysis of liver fat in state of acute hepatic injury caused by ethionine and CCl_4

	fat content %	neutrali. value	iodine value
normal liver	2.7%	165.3	148.5
acute hepatic injury by ethionine	7.9%	185.6	134.7
acute hepatic injury by CCl_4	9.31%	186.4	132.1

2) The Analysis of Liver Fat in Acute Hepatic Injury Caused by the Administration of CCl_4 and Ethionine

The results are shown in Table 15, Fig. 15 and Fig. 16. An increase of the fat content, accompanied by an increase of the neutralization value and a decrease of the iodine value, were both found in hepatic injury, but these changes were less in degree, in comparison with a fatty liver following total pancreatectomy.

Seeing these changes in chromatogram (Fig. 18 and Fig. 19), distinct increase of oleic acid and hexadecenoic acid, and slight trace of lauric acid were found. In these cases also the liver fat had a tendency to become resemble to dépot fat.

According to the results of 1) and 2), it is considered that in an occurance of a fatty liver is probably proceeded by an increase of oleic acid and hexadecenoic acid and then a storing of common saturated higher fatty acids follows. And it is interesting to note that the appearance of lauric acid, which is never found in a normal liver, informates a disturbance of the oxidation mechanism in the liver.

H) Addition: An Analysis of the Fatty Acid Composition of the Adrenal Body in the Dog

The adrenocortical hormone is one of the steroid group, which is synthesized from cholesterol in the living body. And in order to play its physiological role in the living body, the cholesterol must be exist as a fatty acid ester.

From such a point of view, the fatty acid composition of the adrenal body in a normal dog was analyzed.

A healthy adult dog, weighing about 10 kg was sacrificed by bleeding without anesthesia, and immediately the adrenal glands were taken out and analyzed by the same method.

The adrenal gland contained 11.2% fat. It is remarkable that the adrenal gland was very rich in fat, in comparison with other parenchymatous organs. For example, a normal liver contains only about 2.5% fat.

Chromatographic analysis is shown in Fig. 17.

The main characteristic of this chromatogram is its being rich in unsaturated components, especially oleic acid and essential fatty acids (such as linoleic acid, linolenic acid and arachidonic acid) and poor in common saturated higher fatty acids and highly unsaturated acids.

V DISCUSSTION

It has been a well-known theory that fat is an ideal source of nutritions, because in comparison with carbohydrate or protein it has 2 times calories per weight and the lowest specific dynamic action value.

But since there have been many unknown factors in the metabolic process of fat and only the clinical danger of the overeating of fat has been emphasized, oral and parenteral nutritious supplements have not been actively investigated untill recent years. Recently the use of deuterium and radioisotope have brought remarkable advance in this field.

The lipids in our daily food are certainly a complex mixture of neutral fat, free fatty acids, cholesterol, phospholipid and glycolipid, but most part of them is a triglyceride, which is a combination of various fatty acids and glycerine. And these

various fatty acids show different physical and chemical natures according to the number of carbon atoms or their degree of desaturation. For example, in comparing the melting point, solubility in water or acidity, various differences are found. Considering these facts, it is naturally anticipated that when various lipids are uptaken in the living body, they will show several different reactions according to their fatty acid composition.

But for a long time, the only method of analyzing fatty acid composition were the fractional-distillation method of the methyl ester of fatty acid or the fractional-precipitation method of fatty acid salt. Because it was difficult to analyze fat easily and exactly using a small amount of material and because these complicated methods had to be used, fatty acid composition was indirectly presumed from its specific gravity, melting point, refractive index, neutralization value and iodine value. In analyzing fat in the living body, such expessions as "fat having a low melting point" or "highly unsaturated fat" were used and it was rare to discuss the subject using the exact name of each fatty acid.

Recently many contributions have been made in this field by means of various spectrum analysis, molecular distillation method and the analyzing method by urea derivatives. But according to recent progress of chromatography, many excellent methods for the analysis of fatty acid composition were published. Especially, as mentioned above, INOUE, NODA and HIRAYAMA's "Microanalysis of Fatty Acids Mixture by Reversed Paper Chromatography" has aided our study.

According to recent studies about fat absorption in the intestine by using this method, our colleague TAN clarified that all higher fatty acids are absorbed into blood stream through the thoracic duct, while almost all of the lower fatty acids are directly absorbed through the portal vein, as shown by the analysis of blood in the portal vein, and chyle in the thoracic duct after administering various kinds of natural oils. The possibility of having different reactions in the metabolic process after absorption according to the kind of fatty acid, was suggested by the fact that each fatty acid shows a different absorbing mechanism according to the chain length of the carbon atoms.

Actually, ASADA and IZUKURA in our laboratory have used sesame oil emulsion, cod liver oil emulsion and synthetic triolein emulsion for oral and intravenous administration for experimental animals.

The following are the results of micro-histochemical studies in various organs. Injected fat corpuscles left the blood stream within 30 minutes and were phagocytized by the alveolar phagocytes in the lung, KUPFFER's stellate cells in the liver and reticuloendothelial cells in the spleen, and then changed from glyceride to phospholipid in these cells. The phospholipid was shifted to the parenchymal cells in the liver, and it was found that the amount of phospholipids changed according to the kind of fatty acid. When cod liver oil, containing a large amount of highly unsaturated fatty acid, docosenoic acid and eicosenoic acid, or butter, containing lower fatty acids, were given, much larger amounts of phospholipids were found in the parenchymal cells of the liver, than in case of sesame oil, which contains nothing but higher saturated fatty acids, oleic acid and the essential fatty acids.

These facts were confirmed by the experiments of our colleagues SHIROTANI and FUJINO using radioactive P^{32}, and KUYAMA's research on the biochemical changes of phospholipid content in various organs. TOBE, in our laboratory, recently performed the following experiment on the lower fatty acids.

An emulsion of a synthetic simple glyceride mixture, containing equal amounts of tricaproin, tricaprylin and tricaprin, was made, and injected into cats intravenously. Three hours later the cats were sacrificed and the fatty acid content of their various organs, which seemed to have a close relationship to the fat metabolism, such as liver, kidney, lung and heart, were analyzed. It was found that these lower fatty acids had shifted only to the liver, and not at all to other organs. Considering from these experiments, it seems that most of the highly unsaturated fatty acid, docosenoic acid, eicosenoic acid and lower fatty acids are shifted to the liver. On the contrary common higher fatty acids and essential fatty acids seem to be shifted not only to the liver but also to various extrahepatic organs and undergo further metabolism. At this point, the experimental results of this study have confirmed these facts.

After the administration of cod liver oil for a long time, an accumulation of highly unsaturated fatty acid, docosenoic acid and eicosenoic acid was distinctly found in the liver. On the contrary the amounts of these fatty acids, transporting to dépot fat, were extremely less than the amounts of other fatty acids. And at the same time, after the administration of butter, the lower fatty acids were not deposited in dépot fat at all.

The lower fatty acids, which were shifted only to the liver, as shown directly by TOBE's experiment and indirectly by ASADA, and IZUKURA's experiments, were not found at all in the liver 12 hours after feeding, which may be clearly explained by considering that these fatty acids were rapidly oxidized and burned after shifting to the liver.

A definite selectivity was found for deposition of exogenous fat in dépot fat. So when large amounts of all the fatty acids were given continuously more than certain extent, a certain amount was transported into dépot fat, by exceeding the threshold level of the liver. On the contrary it is clear that when sesame oil, containing none of these fatty acids, was administered, even if it was given in large amounts continuously for one month, the liver did not incur any injury, and the rest were transported smoothly into dépot fat and stored there, in preparation for further mobilization.

Formerly many attempts have been made to divide the dépot fat of a certain animal according to its location or kind, and study the differances of each one. HENRIQUES and HAUSEN (1901) have compared the iodine value and melting point of the fat of various animals according to its location, and have pointed out that fat in the superficial layers of adipose tissues is less saturated and has a lower melting point than in deep layers in the body. But, at the same time, this difference has been found to be distinct in big animals, such as horse, sheep or camel, while it is slight in small animals such as dog or rabbit. Then it is considered to

be no mistake that differences were not found in the author's experiment using rats.

Brown fat is one of the adipose tissues which is brown colored and widely located in the neck, scapular, axillar and paraaortic regions and around the thymus gland, kidney and genital organs.

Much attention has been given to it, since it was named "hibernating gland" by RASMUSSEN (1924), because it well developed in hibernating animals, and BUSCHMAN, HOEPKE and NIKOLAUS have agreed with it. But recently many interesting facts have been found by various investigators.

To SCHAEFFER and FEYRTER it seemed as one of the phagocytic organs, and WENDT and HOOK presumed it to be one of the hormonal organs, because decrease of basal metabolism was brought about by administration of brown fat extract.

And metabolism was found to be more accelerated in brown fat than in white fat. This was concluded from the state of fat corpuscles and distribution of blood vessels by FAWCETT, from the conversion rate of radioactive P^{32} by LITTREL and MARTIN and from observation of oxygen consumption by HOOK and BARRON. Also investigation of the components of both adipose tissues has been done by many investigators. Brown fat is richer in cholesterol than white fat by study of CRAMER, and richer in glycogen and female hormone according to a study by HOOKINS and SWEET. Furthermore brown fat is richer in glycogen, cytochrome, cytochrome-oxidase, ascorbic acid, diphosphothiamine, carboxyl group, plasmalogen, cholesterol, phospholipid, acetal dehydrogenase and esterase according to a study by FAWCETT.

WEGENER reported "brown lipom" in man, and HAUSBERGER concluded that brown fat is a premature stage of white fat, according to findings in the embryonal stadium.

But recently experiments using the glucose labeled with C^{14} have considered it an important place for synthesizing glucose to fat. In this study it seems to be no distinct difference between the two adipose tissues by analysis of the fatty acid composition. But a lower content of neutral fat in brown fat shows indirectly to be rich in other components, and few changes of the brown fat ratio to the volume of the total body weight seems to have some meanings.

When the fact that the iodine value of brown fat has a tendency to be less than that of white fat, is considered in agreement with the many opinions that the newly synthesized fat, not having the effect of a desaturating enzyme, is more highly saturated, one evidence seems to be given to the theory that brown fat is the synthesizing place from glucose to fat. But further investigations using another method should be made in order to clarify the essential differences between both adipose tissues and to know the true nature of brown fat.

When the animals are on the verge of starvation, in the early stages glycogen is consumed as a source of energy. After that fat mobilization from dépot fat to liver is accelerated, because at this stage life is maintained chiefly by burning protein and fat. And if starvation continues for a long time the amount of dépot fat in the animal body is greatly decreased, but even if the animal starves to death a great amount of fat can be still found in all the body tissues. Considering these facts, it is clear that there are two kinds of fat in the living body, one of them is

a constant element, playing the role as an essential composing factor of organic substance and not suffering from the influence of the nutritional status, and the other is a variable element, alternating widely according to the nutritional status of the body and being used for the production of energy.

The word "liver fat" has many meanings, one of which is that plays the role as a constant element, while another is that plays as a variable elements, so one cannot treat liver fat simply and indiscriminately. But if after considering these facts sufficiently experiments are performed, as SHAPIRO mentioned, the nature of liver fat may be one of the indicators, representing a state of fat deposition and mobilization. Previously definite selectivities on deposition of exogenous fat in dépot fat and the liver were clarified, and further in order to do research on whether any selectivity on mobilization of dépot fat as a variable element to the liver exists or not and what kind of fatty acid is most easily mobilized, the composition of the liver fat in the state of various experimental fatty livers was analyzed. Of course, there are many causes of fatty liver and many opinions have been mentioned about the etiology of fatty liver, but it may be thought that diabetic fatty liver following total pancreatectomy or fat infiltrations following acute hepatic injury by toxic substances, were chiefly caused by mobilization of dépot fat. Actually according to the results of my experiments at this point, the liver fat composition of a fatty liver distinctly resembles the composition of dépot fat, and it is sufficiently suggested that such mechanism is able to perform in a living body as above mentiond. And in a slight fatty liver, only fat infiltration, accumulation of oleic acid and hexadecenoic acid are most remarkable, but in a typical fatty liver, the appearance of lauric acid and an increase of higher saturated acid with a relative decrease of highly unsaturated acid are shown. The fact that in essential fatty acids, such as linoleic acid or linolenic acid, no remarkable changes are found, suggests their role as a constant element. These mechanisms are also clear on the point of iodine value changes. In the initial stage of a fatty liver a remarkable decrease of iodine value is not found, because the increase of oleic acid and hexadecenoic acid is in balance with the decrease of highly unsaturated acid, but in the latter stages a distinct decrease in iodine value is shown by accumulations of higher saturated fatty acids and a decrease of highly unsaturated acid.

Finally the meanings of my analytic results of the adrenal body, should not be conclusive without many further investigations and examinations. But at least it is clear that the adrenal body contains a large amount of fat, and that fat consists chiefly of cholesterol and essential fatty acids. Considering the fact that cholesterol can be synthesized in a living body but on the contrary essential fatty acids are not synthesized, the important problem will rest in the future with the problem of adrenocortical function.

VI. SUMMARY AND CONCLUSION

These experiments have been performed for the purpose of studying what kinds of fat are most effectively and rationally administered to a living body,

depending upon the investigations of what effects various kinds of exogenously administered fat have on dépot fat and what mechanisms are involved in the fat mobilization from dépot fat. At first, differences between various locations and kinds of adipose tissues (white fat and brown fat) were examined. Then animals were given different kinds of food, containing various natural oils or synthetic glycerides. The fatty acid compositions of these oil were previously known by analysis. And after keeping the animals for one month they were sacrificed, and the neutralization value and iodine value of dépot fat and liver fat were determined, and chromatographic analysis was done at the same time. The analysis of the fatty liver of the dog following total pancreatectomy and analysis of fat infiltration in the liver in acute hepatic injury caused by CCl_4 and ethionine administration, was also done. On the other hand, the fatty acid composition of the adrenal gland of a normal dog was also analyzed, considering the fact that the various functions of fat or essential fatty acid for living body may be performed through adrenocortical hormone.

The following conclusions were obtained:

A) According to the analysis of various natural oils, it was confirmed that butter contains lower fatty acids, such as butyric acid, caproic acid, caprylic acid and capric acid to certain extent, and that cod liver oil contains highly unsaturated fatty acids, docosenoic acid and eicosenoic acid. On the contrary these fatty acids are not found in sesame oil but common higher saturated fatty acids, oleic acid and essential fatty acids are its chief components.

B) Regarding the increase of body weight, the sesame oil diet group was the most excellent. Also the ratio of dépot fat weight to total body weight showed the highest value in the sesame oil diet group.

C) About brown fat, it was presumed that it is not simple dépot fat, since brown fat showed constant location and contained less neutral fat than white fat and the iodine value of brown fat had a tendency to be less than that of white fat. But nothing could be concluded about the nature of brown fat, because its chromatogram had no speciality. Also there were no characteristic effects from the administration of various exogenous fats.

D) A difference in the composition of white fat in the various locations was not found by these analyzing method.

E) Chief components of normal dépot fat were common higher saturated acids such as lauric, myristic, palmitic and stearic acid and unsaturated acids such as oleic, hexadecenoic, linoleic and linolenic acid. On the contrary it was remarkable that in liver fat lauric acid was less than in dépot fat, and it contained a considerable amount of highly unsaturated fatty acid.

F) In the dépot fat of rats, feeding on the butter diet, an increase of lauric acid was found but lower fatty acids less than C_{10} were not found at all.

However, when large amounts of a synthetic simple glyceride mixture of lower fatty acids was given continuously, even lower fatty acids (such as those lower than C_{10}) were deposited in dépot fat. At the same time, these lower fatty acids

were not found in liver fat at all 12 hours after feeding.

G) When the animals were fed on a cod liver oil diet, an accumulation of highly unsaturated fatty acids, docosenoic acid and eicosenoic acid was found in the liver, but deposition of these fatty acids in dépot fat was extremely slight.

H) When the animals were fed on the sesame oil diet, no change was noted in the composition of dépot fat and liver fat.

As shown in F) G) and H), there is a definite selectivity in the deposition of exogenously administered fat in the dépot fat. The highly unsaturated fatty acid, docosenoic acid, eicosenoic acid and lower fatty acids seems to impose a heavy burden on the liver and are not transported smoothly into dépot fat, so it is desirable to select oils, containing as small amount of these fatty acids as possible, when fat is administered into a living body.

I) According to the analysis of a diabetic fatty liver following total pancreatectomy and fat infiltration in the liver in acute hepatic injury caused by CCl_4 or ethionine administration, it was clarified that the fatty acid composition of liver fat resembles that of dépot fat.

J) There is a certain order in the mobilization of dépot fat. It seems that oleic acid and hexadecenoic acid are mobilized first, then lauric acid and other common saturated fatty acids follow.

K) The adrenal gland contained a greater amount of fat than the other parenchymatous organs, and besides cholesterol, the chief components of the fat were oleic acid and the so-called essential fatty acids, such as linoleic acid, linolenic acid and arachidonic acid. This is an interesting fact, suggesting the important role of adrenocortical hormones in connection with the various functions of fat to the living body or important nutritious meanings of essential fatty acids, which have been previously known.

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REFERENCES

1) Akamatsu, S.: Shinpan Seikagaku. Kyoritus Press, Tokyo, 1956.
2) Anker, H. S.: On the metabolism of fatty acid synthesis in vivo. J. Biol. Chem., **194**, 177, 1952.
3) Aoki, H.: A contribution to the problem of fatty liver following total pancreatectomy. Laboratory studies in dogs and men. Arch. Jap. Chir., **23**, 203, 1954.
4) Artom, C., Cornatzer, W. E.: The action of choline and fat on lipide phosphoration in the liver. J. Biol. Chem., **171**, 779, 1947.
5) Asada, S.: Histochemical studies on the intravenously infused fat emulsion. Arch. Jap. Chir., **22**, 77, 217, 1954.
6) Baker, B. L., Ingle, D. J., Chon, H. L.: Increase in glyceride content of brown fat by treatment with adrenocorticotropin. Proc. Soc. Exp. Biol., **73**, 337, 1950.
7) Bloom, B., Chaikoff, J. L., Reinhardt, W. O., Dauben, W. G.: The quantitative significance of the lymphatic pathway in transport of absorbed fatty acid. J. Biol. Chem., **184**, 1, 1950.
8) Bloor, W. G.: Biochemistry of the fatty acid. Reinhold, New York, 1943.
9) Burr, G. O., Burr, M. M.: On the nature and role of the fatty acids essential in nutrition. J. Biol. Chem., **86**, 587, 1930.

10) Cameron, G. R., Karunaratne, W. A. E.: Carbon tetrachloride cirrbosis in relation to liver regeneration. J. Path. Bact., **42**, 1, 1936.
11) Chaikoff, I. L., Enterman, C., Montgomery, M. L.: The mechanism of action of the antifatty liver factor of the pancreas. J. Biol. Chem., **168**, 1947.
12) Chaikoff, I. L., Entenman, C.: Antifatty liver factor of the pancreas. Advances in Emzymolomegy. **8**, 171, 1948.
13) Clément, G.: Mobilisation of reserve glycerides in the rat. Difference in the mobilisation provoked by fact and by thermoregulation. Arch. Soc. Physiol., **4**, 1, 1950.
14) Cramer, W.: On glandular adipose tissue and its relation to other endocrine glands and to vitamine problem. Brit. J. Exp. Path., **1**, 184, 1920.
15) Dermann, G. L., Leites, S.: Experimental-morphologische Studien über die Rolle der Lunge, Leber, u. Milz in Fett u. Lipoid-Stoffwechsel. Virchow's Arch., **264**, 440, 1928.
16) Elaine, P. R., Saul, H. R.: The effect of meat and meat fractions on the fatty liver of the depancreatized and pancreatic-duct ligated dog. Am. J. Phisiol., **138**, 42, 1942.
17) Ernest, B.: Dynamic aspects of biochemistry. Cambridge University Press. 1953.
18) Farber, E., et al.: Studies on ethionine.][. The inference with lipid metabolism. J. Biol. Chem., **182**, 91, 1950.
19) Fawcett, D. W.: Differences in physiological activity of brown and white fat as revealed by histochemical relations. Sciense, **105**, 123, 1947.
20) Fawcett, D. W.: Histological observation on the relation of insulin to the deposition of glycogen in adipose tissue. Endocrinology, **42**, 454, 1948.
21) Fawcett, D. W.: A comparison of the histological organisation and cytochemical reactions of brown and white adipose tissue. J. Morph., **90**, 363, 1952.
22) Fawcett, D. W., Jones, I. C.: The effects of hypophysectomy, adrenalectomy and of thiourocil feeding on the cytology of brown adipose tissue. Endocrinology, **45**, 609, 1949.
23) Geyer, R. P., Marry, C.: Metabolism of fatty acids in vitro, studied with odd and even members of the $RC^{14}OOH$ series. J. Biol. Chem., **184**, 641, 1950.
24) Goldmann, D. S., Chaikoff, I. L., et al.: The oxidation of palmitic acid 1-C^{14} by extrahepatic tissues of the dog. J. Biol. Chem., **184**, 719, 1950.
25) Goldman, D. S., Chikoff, I. L., et al.: Site of formation of phospholipids studied with C^{14}-labeled palmitic acid. J. Biol. Chem., **184**, 727, 1950.
26) Gorens, S. W., Geyer, R. P., et al.: Parenteral nutrition. X. Observation of the use of a fat emulsion for intravenous nutrition in man. J. Lab. Cli. med., **34**, 1627, 1949.
27) Grafflin, A. L., Green, D. E.: Studies on the cyclophorase system.][. The complete oxydation of fatty acid. J. Biol. Chem., **176**, 95, 1948.
28) Hashino, H.: Experimental studies of fat metabolism from the viewpoint of ketone body formation. Arch. Jap. Chir., **24**, 488, 1955.
29) Hikasa, Y., et al.: Shishitushokyu no Kiso to Zissai. Shinryo, **11**, 922, 1041, 1958.
30) Hikasa, Y.: Shiboyueki ni kansuru Syomondai. Gekakenkyu no Shinpo, **2**, 15, 1957.
31) Hikasa, Y.: Shishitusnyuzai o mottesuru Shishitsutaisya narabini sono Eiyogakuteki Igi ni tsuiteno Kenkyu. Saishinigaku, **13**, 2278, 2586, 2954, 1958.
32) Hikasa, Y., Asada, S., Zaitsu, A., Tsukata, A., Nakata, K.: Studies on the intravenous administration of fat emulsion. Arch. Jap. Chir., **21**, 1, 1952.
33) Hikasa, Y., Ishigami, K., Asada, S., Zaitsu, A., Tsuata, A., Nakata, K.: Studies on the intravenous administration of fat emulsion. J. J. S. S., **52**, 298, 1951.
34) Hikasa, Y.: Studies on the intravenous administration of fat emulsion prepared by high pressure jet. Rev. Phys. Chem. Jap., **12**, 83, 1952.
35) Hikasa, Y., Hashino, H., Osa, H., Takeda, S.: The role of water soluble vitamine in fat metabolism. Nipponrinshi, **13**, 1225, 1955.
36) Hirao, N.: Yushikagaku Honron. Kazamashobo, Chiba, Japan, 1948
37) Hilditch, T. P.: The chemical constitution of natural fats. Chapman and Holl, London, 1947.
38) Hook, W. E., Barron, E. S. G.: The respiration of brown adipose tissue and kidney of the hibernating and non-hibernating ground squirrel. Am. J. Physiol., **133**, 56, 1941.
39) Hirose, S.: Cytological study on the fatty liver caused by feeding high fat diet. Arch.

Hist. Jap., **7**, 513, 1955
40) Ikeda, H.: Experimental studies on fat metabolism with a blocked reticuloendotherialsystem. Arch. Jap. Chir., **26**, 355, 1957.
41) Inoue, Y., Hirayama, O., Noda, M.: Separation and identification of fatty acids. XX. 2,4-dinitrophenylhydrazones of p-bromphenacylesters as derivatives for characterisation of unsaturated fatty acids. Bull. Agr. Chem. Soc. Japan, **20**, 4, 197, 1956.
42) Inoue, Y., Hirayama, O., Noda, M.: Separation and identification of fatty acid. XXI. paperchrormatography of fatty acids as their p-bromphenacylester derivatives. Bull. Agr. Chem. Soc. Japan, **20**, 4, 200, 1956.
43) Ito, T.: Studies on the "fat storing cells" in the liver. Kaibo, Z., **31**, 10, 1956.
44) Izukura, T.: Histochemical studies on intravenously administrated fat emulsion. Arch. Jap Chir., **27**, 14, 1958.
45) Jaffé, R. H., Berman, S. L.: The relations between Kuffer cells and liver cells. Arch. Pathol., **5**, 1020, 1928.
46) Jeckeln, E.: Über der Rolle der Lungen beim Fettstoffwechsel. Ziegler's Beitr., **92**, 357, 1933.
47) Kaplan, A., Chaikoff, I. L.: Liver lipids in completly depancreatized dogs maintained with insulin. J. Biol. Chem., **108**, 201, 1935.
48) Kimura, S.: Histologische Untersuchungen über das Schicksal intravenöse infundierten Fettes in Organismus. Tohoku J. Exp. Med., **30**, 315, 1937.
49) Koch, W. D., Fauber, E., Popper, H.: Fatty liver with and without necrosis.: Histological and biochemical investigations. Arch. Pathol., **51**, 498, 1951.
50) Kotake, M.: Shinpen Yukikagaku Zikkenho. Rikoshuppansha, Tokyo, 1953.
51) Kuwata, S.: Chromatography. Hirokawashoten, Tokyo, 1956.
52) Kuyama, T.: Clinical studies on the nutritial effects of intravenous administration of fat emulsion. Arch. Jap. Chir., 27, 64, 1958.
53) Littrell, J. L., Martin, C. G.: Phosphorus turnover in brown fat as demonstrated by radioactive phosphorus. Anat. Rec., **89**, 39, 1944.
54) Mason, K. E., Dam, H., Granodos, H.: Histological changes in adipose tissue of rats fed vitamine E deficient diet high in cod liver oil. Anat. Rec., **94**, 265, 1946.
55) Matsui, C.: Studies on alkaline phosphatase value in damaged liver. Jap. Arch. Int. Med., **4**, 107, 1957.
56) Matsushita, K.: Experimental studies in the influences of vitamins of B group and of methionine upon the fat content of the liver. Jap. Arch. Int. Med., **3**, 566, 569, 1956.
57) Mckibbin, J. M., Stare, F. S.: Studies of fat emulsion for intravenous alimentation. J. Lab. Kli. Med., **30**, 488, 1945.
58) Meng, H. C., Freeman, S.: Experimental studies on the intravenous injection of a fat emulsion into dogs. J. Lab. Cli. Med., **33**, 689, 1946.
59) Menshick, Z.: Histological comparison of brown and white adipose tissue in guinea pigs. Anat. Rec., **116**, 439, 1953.
60) Mickelwright, L.: Glycogen deposition in the liver and in the intrascapular fat body of the mouse. Fed. Proc., **9**, 89, 1950.
61) Mirski, A.: The metabolism of adipose tissue in vitro. Biochem. J., **36**, 232, 1942.
62) Munoz, J. M., Lelion, L. F.: Fatty acid oxidation by liver enzymes. J. Biol. Chem., **147**,355, 1953.
63) Nishimura, T.: Morphological studies on fat metabolism and its disorders. Nippon Taishitsu Igaku Zashi. **21**, 101, 1956.
64) Nishino, T.: Laboratory studies on the intravenous administration of the fat emulsion in the light of tissue metabolism. Arch. Jap. Chir., **23**, 607, 1954.
65) Noda, M., Hirayama, O.: Metalic addition compounds of unsaturated fatty acids. II. Micro-analysis of unsaturated fatty acid esters by mercuration method. Scient. Rep. Saikyo, Univ., **6**, 86, 1954.
66) Noda, M., Hirayama, O.: Paperchromatogrphy of fatty acids as their acetol ester derivatives. Scient, Rep. Saikyo Univ., **7**, 43, 1955.
67) Okuzono, H.: Research of fatty liver. Kumamoto, Med. J., **8**, 73, 1955.

68) Ōji, K., Wada, M.: Fat metabolism in the liver. Sōgōrinshō, **4**, 1050, 1955.
69) Ōmura, Y., Osaka, Y.: Salt solution of multi water soluble fatty acid for injektion as a nutritial transfusion materials of fatty substance. J. J. S. S. **56**, 779, 1955.
70) Ono, K.: Fat metabolism. Saishinigaku, **10**, 52, 1955.
71) Ono, K.: Shokujisei Shibo oyobi soreni kanrenseru Shoshikkan ni tsuite. Shischitsu tokuni Rinshischitsu-taisha o Chushin to shite. Nisshinigaku, **14**, 4. 197, 1957.
72) Osa, H.: Experimental studies on the intravenous administration of a fat emulsion for nutritial purpose. Arch. Jap. Chir., **25**, 154, 1956.
73) Ōtani, S.: On mechanism of adverse reaction caused by intravenous administration of fat emulsion. Arch. Jap. Chir., **25**, 172, 1956.
74) Quastel, J. H., Wheatley, A. H. M.: Oxidation of fatty acids in the liver. Biochem. J., **27**, 1753, 1933.
75) Rasmussen, A. T.: The "so-called hibernating gland. J. Morph., **38**, 147, 1924.
76) Reiser, R., Bryson, M. J.: Route of absorbtion of free fatty acid and triglycerides from the intestine. J. Biol. Chem., **189**, 87, 1951.
77) Renold, A., Marble, A., Fawcett, D. W.: Action of insulin on deposition of glycogen and storage of fat in adipose tissue. Endocrinology, **46**, 55, 1950.
78) Selye, H.: The story of the adaptation symdromes. Acta Press, Montriol. Canada, 1952.
79) Seno, A.: A study of fat metabolism in the isolated perfused liver. Arch. Jap. Chir., **24**, 179, 1955.
80) Shafiroff, B. G. P., Frank, C.: A homogenous emulsion of fat, protein and glucose for intravenous administration. Science, **106**, 474, 1947.
81) Shafiroff, B. G. P., Hulholland, J. H.: Effects of intravenous infusion of experimental "instant" fat emulsion into volunteer subjects. Proc. Soc. Exp. Biol. Med. **91**, 111, 1956.
82) Sheldon, E. F.: The so called hibernating gland in mammals, a form of adipose tissue. Anat. Rec. **28**, 331, 1924.
83) Shorland, F. B.: Prog. in the chemistry of fat and other lipids. Pergamon Press, London, **3**, 275, 1955.
84) Schoenheimer, R., Rittenberg, O.: Deutrium as an idnicator in the study of intermediary metabolism. Ⅲ. the role of fat tissue. J. Biol. Chem. **111**, 175, 1935.
85) Schoenheimer, R., Rittenberg, O.: Deutrium as an indicator in the study of intermediary metabolism. Ⅸ. The conversion of stearic acid into palmitic acid in the organismus. J. Biol. Chem., **120**, 155, 1937.
86) Shirotani, H.: Histochemical studies on fat metabolism by intravenous administration of fatty chyle. Arch. Jap. Chir., **26**, 38, 1957.
87) Sideman, R. L., Fawcett, D. W.: The effect of peripheral nerve section on some metabolic responses of brown adipose tissue in mice. Anat. Rec., **118**, 3, 487, 1901.
88) Takeda, I.: Supplemental informations of the changes in the cholesterol content of blood serum in hepatic injury. Jap. Arch. Int. Med., **4**, 274. 1957.
89) Tan, N.: Analytic chemical studies on fat metabolism by application of paper chromatography of fatty acids. Arch. Jap. Chir., **28**, 1178, 1959.
90) Tatsumi, W.: Klinische Beobachtungen über die Intravenöse Infusion des Fettes. Arch. Jap. Chir., **26**, 1, 1957.
91) Tobe, R.: Experimental investigation of various fats as to their nutritional value. Arch. Jap. Chir., **28**, 184 1959.
92) Tsuchiya, T.: Zitsuyo Yushibinran. Sangyotosho, Tokyo. 1956.
93) Tsukata, A.: Studies on the fat emulsion in the light of protein metabolism. Arch. Jap. Chir., **23**, 215, 1954.
94) Wool, L. G., Goldstein in M. S.: Role of neurohumors in the action of the adrenal cortical steroids: Mobilisation of fat. Am. J. Physiol. **175**, 303, 1953.
95) Yamada, M.: Relations between liver fat and dietary conditions. Handai Ishi, **3**,37,181,1951.
96) Yasuda, M.: Fat metabolism. Nishinigaku, **32**, 501, 1943.

和文抄録

脂酸ペーパークロマトグラフィーによる各種組織の分析化学的研究

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著者は各種の組成を有する外来脂質が，生体の貯蔵脂肪に如何なる影響を与えるかという点と，貯蔵脂肪からの脂肪の動員が如何なる機序で行われるかという点を追究し，それによつて，如何なる組織をもつた脂肪を投与するのが，生体にとつて最も有効且つ合理的であるかを追究する目的で，本実験を行つた．

まずラットを用いて，貯蔵脂肪の分布場所及び種類（白色，褐色）による差違を検討した後，分析によつて組成を明らかにした各種の油脂及び合成トリグリセライドを含む食餌を1ヵ月に亘つて投与し，その貯蔵脂肪及び肝脂肪について，中和価及び沃素価を測定すると同時に，又クロマトグラフィーをも併せ行い，その成分分析をも行つた．

更に，膵臓全剔犬の脂肝及び四塩化炭素，エチオニンによる急性肝障害を惹起したラットの肝組織について，同様の分析を行つた．

また別に，脂肪あるいは不可欠脂酸の生体に及ぼす各種の作用が，副腎皮質ホルモンを介して行われるのではないかとの推察のもとに，正常犬の副腎脂肪をも分析した．

1） 各種の油脂の分析によつて，バターには酪酸，カプロン酸，カプリル酸，カプリン酸等の低級脂酸がある程度含有され，肝油には高度不飽和酸及びドコセン酸，エイコセン酸が含有されているのに対し，ゴマ油にはこれらのものはなく，一般高級飽和脂酸，オレイン酸，リノール酸，リノレン酸が主成分となつていることを確認した．

2） 体重増加の面からいえば，ゴマ油が最も優れて居り，体重に対する貯蔵脂肪の重量比からいつても，ゴマ油飼育群が最高である．

3） 褐色脂肪について，その分布状態の恒常性及び中性脂肪の含有率の少いことから，またその沃素価が白色脂肪よりやゝ低い傾向にあることから，単なる貯蔵脂肪でないことが憶測出来た．併しクロマトグラムには特徴がなく，何等結論的なことはいえない．また各種外来脂肪による影響にも特徴的なことは毫も認められない．

4） 白色脂肪の分布場所による組成の差違は，この分析法を以てしては認められない．

5） 正常の貯蔵脂肪の主成分はラウリン酸，ミリスチン酸，パルミチン酸，ステアリン酸の各一般高級飽和脂酸と，オレイン酸，ヘキサデセント酸及びリノール酸，リノレン酸等の不可欠脂酸であり，肝脂肪はこれに対し，ラウリン酸が認められず，オレイン酸，ヘキサデセント酸が少く，高度不飽和酸をかなり多量に含有することが特徴である．

6） バター食による飼育ラットの貯蔵脂肪にはラウリン酸の増量がみられるが，C_{10} 以下の低級脂酸は認められない．併しC_{10}以下の脂酸の Simple glyceride mixtare を合成して，大量に且つ持続して投与すれば，C_{10} 以下の低級脂酸でも，貯蔵脂肪中に沈着される．但しこの際，食後12時間では，肝脂肪に C_{10} 以下の低級脂酸は認められなかつた．

7） 肝油食による飼育群では，肝臓に高度不飽和酸，ドコセン酸，エイコセン酸の貯溜がみられたが，これらの脂酸の貯蔵脂肪への移行は極めてわずかである．

8） ゴマ油食による飼育群では，貯蔵脂肪及び肝脂肪に格別の成分変化を来たさない．

6)7)8)に示すように，外来脂肪の貯蔵脂肪中への沈着には一定の撰択性があり，高度不飽和酸，ドコセン酸，エイコセン酸及び低級脂酸は，肝臓に負担をかけスムースに貯蔵脂肪中に移行しないから，生体に対する脂肪補給に際しては，これらの脂酸の含有率の少い脂肪を選ぶことがのぞましい．

9） 膵全剔犬の糖尿病性脂肝及四塩化炭素，エチオニンによる急性肝障害によつて脂肪浸潤を来した肝臓を分析して，かかる場合の肝脂肪の組成が，貯蔵脂肪のそれに近似していることが明らかとなつた．

10） 貯蔵脂肪の動員にも一定の順序があり，オレイン酸，ヘキサデセント酸がまず動員され，次いでラウ

Fig. 1 Chromatogram of fatty acid in sesame oil, cod liver oil and synthetic triglyceride mixture

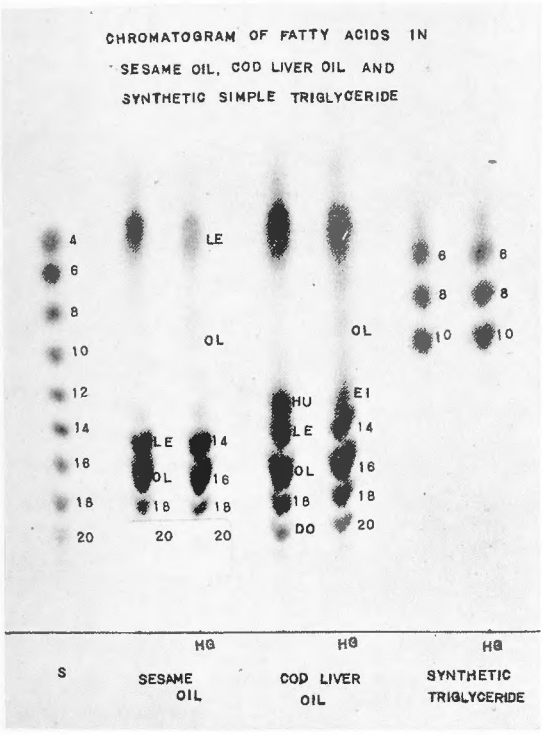


Fig. 2 Chromatogram of fatty acid in butter

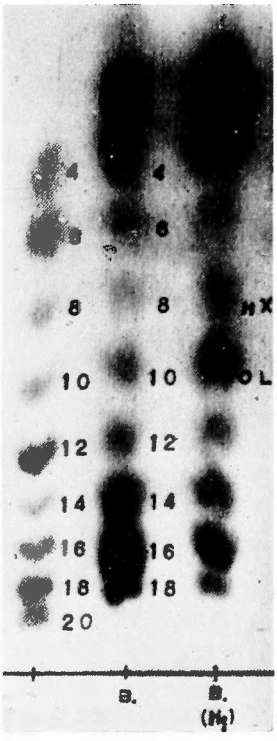
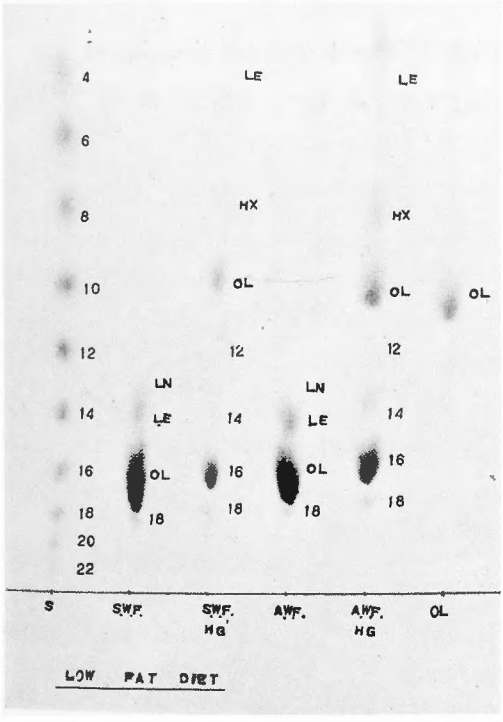
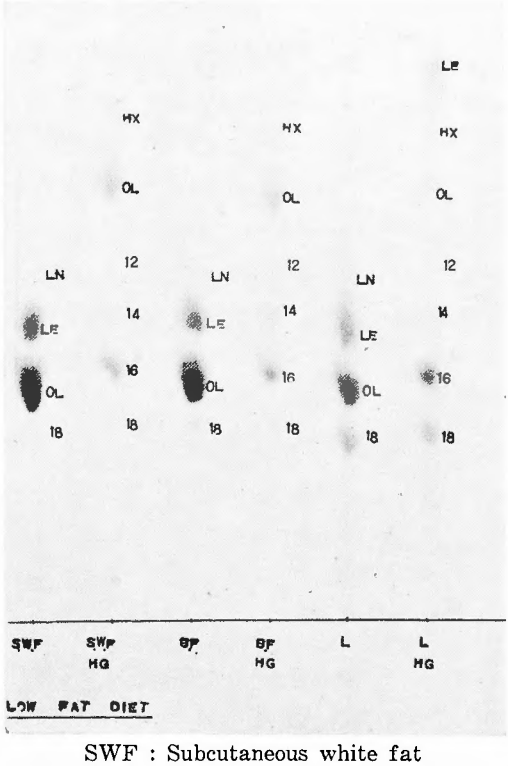


Fig. 4 Chromatogram of fatty acid in various adipose tissues and liver in low-fat diet group

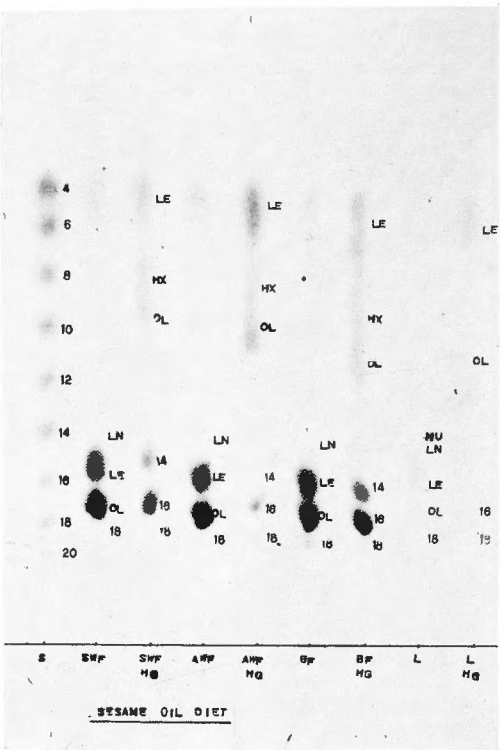




S : Sample
BF : Brown fat
AWF : Abdominal white fat

SWF : Subcutaneous white fat
L : Liver

Fig. 7 Chromatogram of fatty acid in various adipose tissues and liver in sesame oil diet group



S : Sample

Fig. 8 Chromatogram of fatty acid in various adipose tissues and liver in cod liver oil diet group

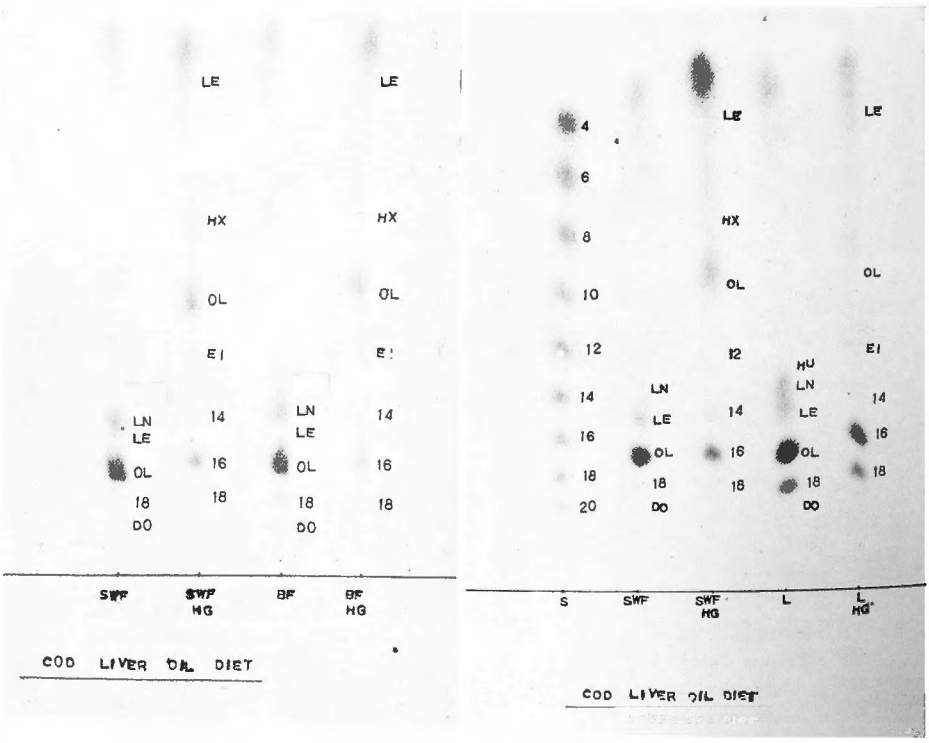


Fig. 9 Chromatogram of fatty acid in various adipose tissues and liver in butter diet group

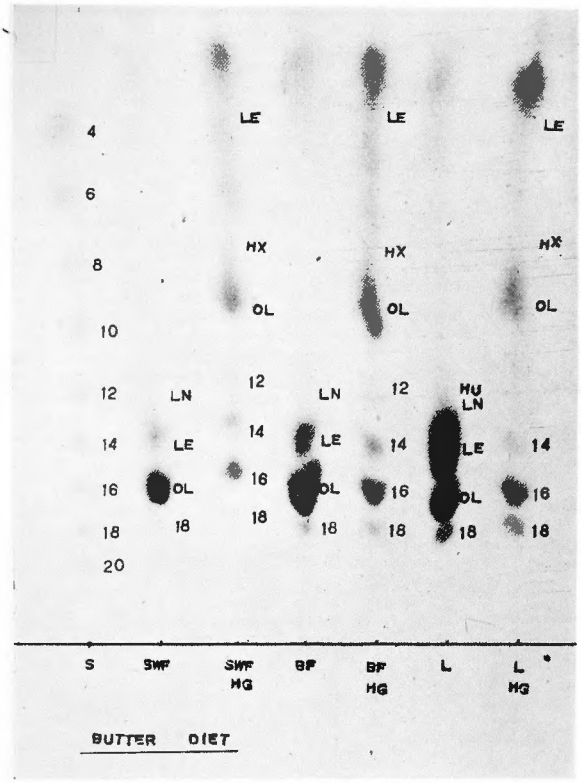
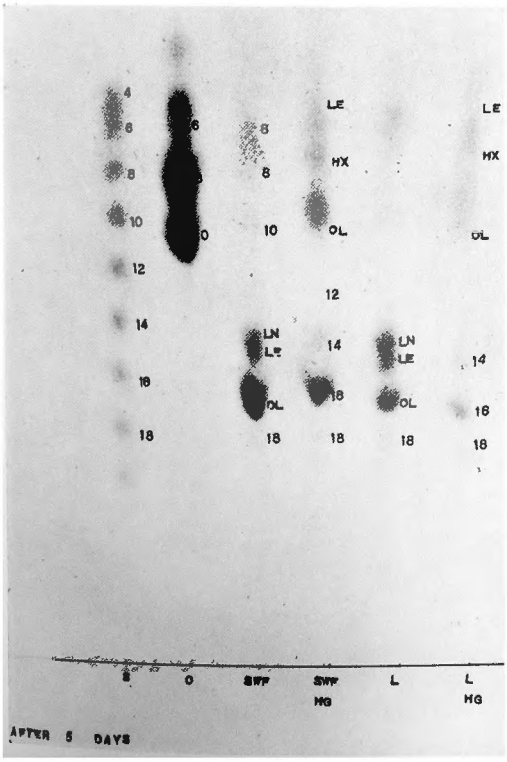
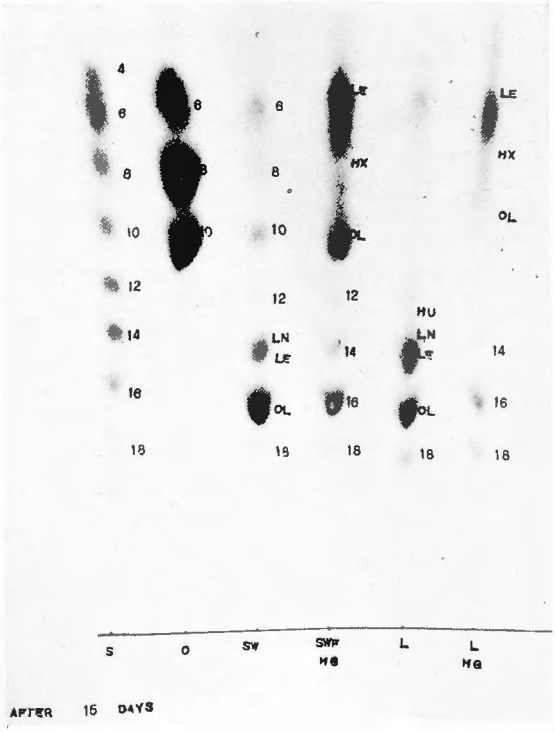


Fig. 12 Chromatogram of fatty acid in dépot fat and liver in lower fatty acids diet group

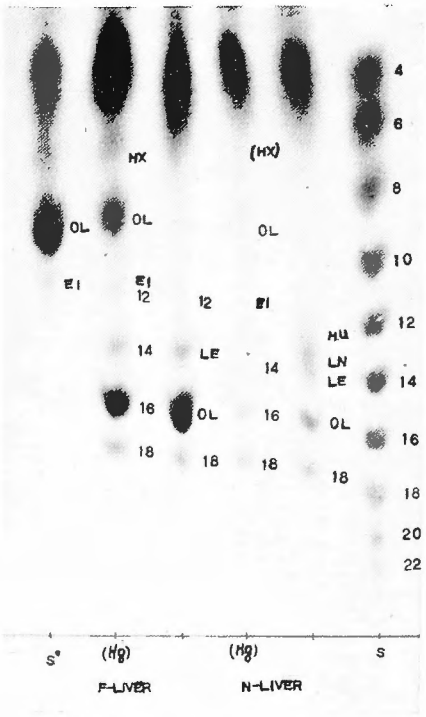


after 5 days



after 15 days

Fig. 13 Chromatogram of fatty acid in fatty liver following total pancreatectomy



S : Sample (saturated acids of even number)
S′ : Sample (Oleic acid and eicosenoic acid)
N-Liver : normal liver
F-Liver : fatty liver

Fig. 14 Chromatogram of fatty acid in subcutaneous fat of normal dog

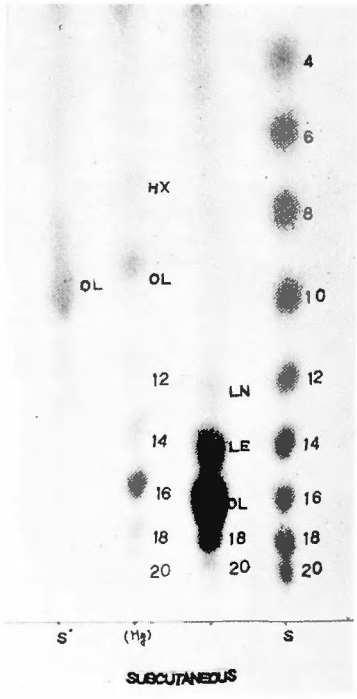
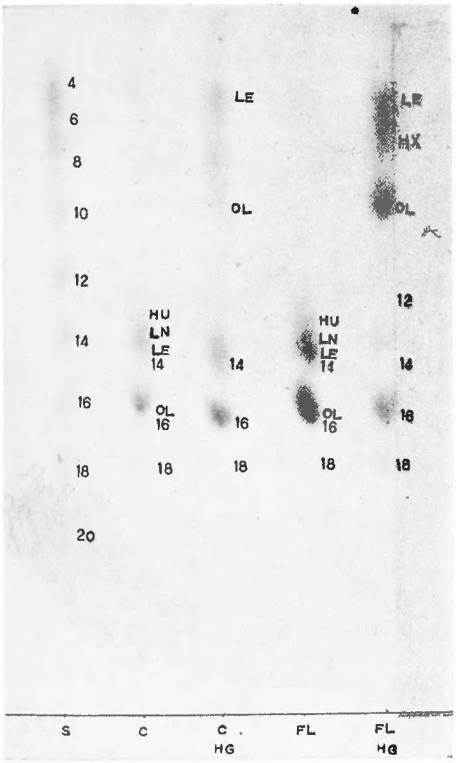
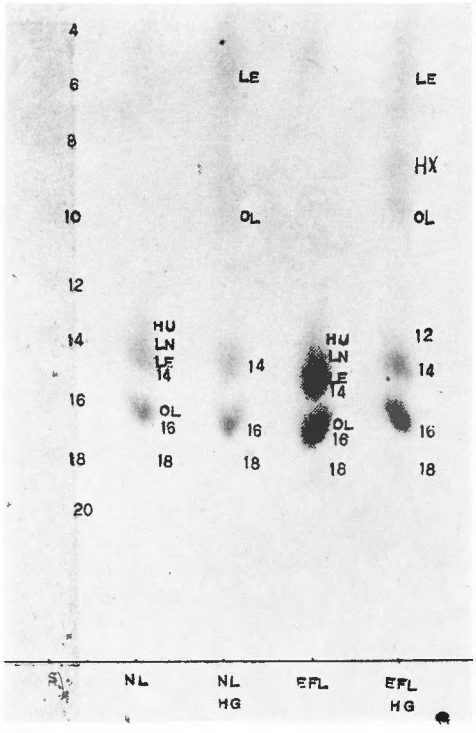


Fig. 15 Chromatogram of fatty acid in liver in state of acute hepatic injury caused by CCl_4



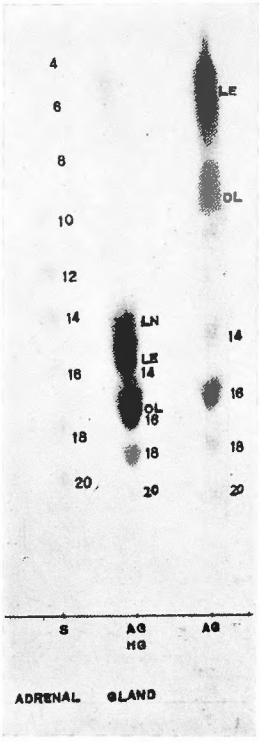
C : control (normal liver)
FL : fatty liver by CCl_4

Fig. 16 Chromatogram of fatty acid in liver in acute hepatic injury caused by ethionine



NL : normal liver
EFL : fatty liver by ethionine.

Fig. 17 Chromatogram of fatty acid in adrenal gland of normal dog



リン酸を初めとする一般飽和脂酸が動員されるものと考えられる.

11) 副腎の脂肪含有量は，他の実質臓器に比して非常に多く，而もその脂肪の主成分をなすものは，コレステロールを除けば，オレイン酸及びリノール酸，リノレン酸等の所謂不可欠脂酸である．このことは，すでに明らかにされた脂肪の生体に対する諸作用，ひいては不可欠脂酸の栄養学的意義に関して，副腎皮質ホルモンの占める役割の重要性を示唆する興味ある事実である.